Kobe J. Med. Sci., Vol. 54, No. 1, pp. E62-E72, 2008

Effect of Ground Green Tea Drinking for 2 Weeks on the Susceptibility of Plasma and LDL to the Oxidation *ex vivo* in Healthy Volunteers.

SYUZOU GOMIKAWA, YUICHI ISHIKAWA, WAKAKO HAYASE, YUKO HARATAKE, NAMIKO HIRANO, HIROMI MATUURA, AKIYO MIZOWAKI, AKIKO MURAKAMI, and MAO YAMAMOTO

Faculty of Health Sciences, Kobe University School of Medicine, Kobe 654-0142, Japan.

Received 17 August 2007 /Accepted 10 December 2007

Key words: green tea, catechins, antioxidants, LDL

Catechins in green tea have been shown to reduce a risk of coronary heart disease in epidemiological studies. Also, it has been reported catechins have hypolipidemic and antioxidant effects. Then, we investigated the effects of ground green tea drinking on the susceptibility of plasma and low-density lipoprotein (LDL) to the oxidation by $CuSO_4$ ex vivo, and also evaluated daily food consumption using semiquantitative questionnaire. Five healthy female subjects consumed ground green tea (1.5 g/ 3 times/ day) for 2 weeks after a washout period of 1 week, when they drank water instead of tea. After 2-week tea drinking, the subjects drank water again. They also filled food and drink-frequency questionnaires during 4 weeks to assess daily foods consumption to estimate the oxidizability of plasma and LDL. We measured the lag time of conjugated dienes formation of plasma and LDL to oxidation by $CuSO_4$. The lag time of conjugated dienes formation are increased in all subjects after ground green tea consumption from 67± 19 to 118± 42 min in plasma and from 47± 6 to 66± 10 min in LDL. The cholesterol contents in plasma and LDL decreased 10 mg / dl after ground green tea consumption. The β-carotene, α-tocopherol, vitamin C and uric acid contents in plasma did not change after ground green tea consumption. The superoxide dismutase (SOD) activity in plasma also remained unchanged during this study periods. These findings indicated that ground green tea consumption decreased susceptibility of plasma and LDL to oxidation and also modulated cholesterol metabolism and might prevent initiation and progression of atherosclerosis

Green tea (*Camellia sinensis*) is a widely consumed beverage in the world, and contains antioxidants such as catechins, ascorbic acid, α-tocopherol and β-carotene [9]. Epidemiological studies have reported a reduced risk of coronary heart disease in subjects with a high flavonoid intake through tea and other dietary sources [7, 12, 15], but findings are still conflicting [28]. Also, two conflicting epidemiological studies have reported on the association of green tea consumption and serum cholesterol level [36, 37]. These major mechanisms have been considered; hypolipidemic effect [1, 3, 21, 22, 25, 39, 40, 43], antioxidant effect [8, 42], antihypertensive effect [27] and other effects [11, 16]. It has been reported that catechins in green tea inhibited cholesterol absorption in intestine of rats [22, 25], the activities of lipids enzymes [1, 21, 39, 40] and the apoB secretion in HepG-2 cells [43] and also increased LDL receptor [3]. Also, the catechins are group of polyphenolic compounds; they have been shown to possess strong antioxidant properties for plasma and

Phone : 078-796-4512 Fax : 078-796-4512 E-mail: yishikaw@kobe-u.ac.jp

LDL oxidation *in vitro* and *ex vivo* [8, 42]. Yamanaka et al. [42] showed that (-) epicatechin (EC) and (-) epigallocatechin (EGC) had a strong inhibitory effect of LDL oxidation *in vitro*. We also reported the inhibitory activities of EC and (-) epigallocatechin gallate (EGCg) on LDL oxidation by $CuSO_4$, and the antioxidant activity of EGCg to LDL oxidation was stronger than that of EC *in vitro* [8]. As other effects, catechins have been shown to exert pharmacological effects including antiproliferation [11], and antiadhesion [16]. Therefore, the consumption of tea catechins may reduce plasma cholesterol, susceptibility to oxidation and the risk for coronary heart disease. However, there are not always consisted with the reports about antioxidant *ex vivo* [13, 16, 38] and hypolipidemic effects [23, 44] of the catechins in human.

Then, we investigated the effects of pulverized ground green tea drinking on the susceptibility of plasma and LDL to oxidation by $CuSO_4$, and on cholesterol concentration in plasma in healthy Japanese females and also evaluated daily food consumption using semiquantitative questionnaire [35].

MATERIALS AND METHODS

Materials

(+) catechin (CA), EC, (-) epicatechin gallate (ECg), EGC, EGCg, sulfatase (EC 3.1.6.1, Type VIII: from Abalone Entrails, S-9754) and β-glucuronidase (EC 3.2.1.31, Type X-A, from *E. Coli*, G-7896) were obtained from Sigma chemical (St. Louis, Mo., USA). Ground green tea (senncha) was obtained from Ujimoritoku (Mathubara, Osaka, Japan). Acetonitril, ethyl acetate, N,N′-dimethylformamide and all other chemicals were purchased from Wako Pure Chemical Industries (Osaka, Japan).

Human subjects

All subjects gave oral informed consents for study participation before experiments. All subjects were nonsmoking volunteers. The characteristics of five healthy female volunteers in this study were as follows; age 21±1 years old; body weight 50± 4 kg; height 158± 6 cm; body mass index (BMI) 20±1 Kg /m^2. Blood chemical tests were as follow: plasma Triglycerides 67±37 mg/dl; Total cholesterol 187±33 mg/dl; LDL-cholesterol 104±32 mg/dl; HDL-cholesterol 68±15 mg/dl; phospholipids 221±29 mg/dl; aspartate aminotransferase (AST) 17± 1 IU/l; alanine aminotransferase (ALT) 12± 3 IU/l.

Study design

After a washout period of 1 week without tea drinking, subjects consumed ground green tea (1.5 g/ 3 times/ day) for 2 weeks. During the next 1 week, the subjects drank water daily. In addition to no-tea drinking, subjects were not allowed to consume alcoholic beverages, antioxidant vitamins (ascorbic acid, α-tocopherol, carotene), chocolate and more than 2 fruits /day during the intervention. All subjects also were directed not to change daily eating and drinking habits other than instructions. For the confirmation, we examined food and drink intakes about each subjects with a semiquantitative food frequency questionnaire during all experimental periods [35].

Blood sampling

Venous blood samples were collected in tube containing 1mg / ml EDTA from subjects after 12 h fasting each weeks. Plasma was prepared by low speed centrifugation (3,000 r.p.m., 10 min.) within 30 min after blood sampling.

LDL preparation

LDL ($1.025<d<1.050$, 541,000×g, 2 h, 4 ^oC) was obtained by preparative ultracentrifugation by methods described previously [8]. Isolated LDL was dialyzed against 0.01 M phosphate buffer (pH 7.4), 0.16 M NaCl and 0.2 μM EDTA (PBS-EDTA) saturated

with N_2 gas for 20 h at 4 °C. We confirmed purity of LDL using SDS-polyacrylamide gel electrophoresis according to the method of Graham [10].

Susceptibility of plasma and LDL to oxidation *ex vivo*

The methods of susceptibility of plasma and LDL were previously described elsewhere [8]. Briefly, the effect of consumption of green tea on plasma oxidation *ex vivo* was determined using the method of Kontush et al [17]. Plasma sample were incubated at 37 °C in the presence of PBS-EDTA buffer containing 50 μM $CuSO_4$ and conjugated dienes production was measured by the changes of the absorbance at 234 nm using a Shimadzu Model UV-1600 PC spectrophotometer. The lag time of LDL to oxidation by 5 μM $CuSO_4$ was determined using the method of Esterbauer et al [4].

Analysis of catechins contents in ground green tea and plasma

The catechins contents in ground green tea were determined using previously described method. The ground green tea (n=6) used in this study contained CA of 7.32 ±0.43 mg/g, EC of 15.5±2.7 mg/g, ECg of 20.6±3.3 mg /g, EGC of 51.2±2.7 mg/g, EGCg of 87.6±11.2 mg/g and total catechins of 183±13 mg/g. The catechins concentrations in plasma were determined using previously described method [8]. Briefly, plasma sample were incubated with a mixture of β-glucuronidase and sulfatase, and the free form of catechins were obtained. After extraction into ethyl acetate, the catechins were analyzed by HPLC.

Chemical analysis in plasma

Plasma cholesterol, triglycerides and phospholipids concentrations were determined using enzymatic colorimetric methods [8]. HDL-cholesterol, LDL-cholesterol and uric acid were measured enzymatically by commercially available kit (Daiichi Pure Chemicals Co., LTD, Tokyo). Vitamin C, α-tocopherol and β-carotene concentrations in plasma were measured by HPLC [2, 14, 34]. Plasma thiobarbituric acid reactive substances (TBARS) were determined by fluorometric assay [41]. Superoxide dismutase (SOD) activity in plasma was measured by commercially available kit (Wako Pure Chemical Industries, Osaka, Japan).

α-tocopherol and β-carotene analysis in LDL

α-tocopherol and β-carotene in LDL were extracted with ethanol : hexane (10 : 1), and the extracts were concentrated by N_2 gas. The concentrated sample was then dissolved with the movile solvent for HPLC analysis. The HPLC analysis was performed using the method of Shidouji [33] with some modification: apparatus, Model 802 (Gilson Co, Ltd., Middleton, WI, U.S.A.), UV-master Model 1001 (M and S Instrument, Osaka, Japan), column Develosil ODS-80Ts (4.6×250 mm: TSK gel, TOSO CO., Japan), eluent (methanol: butanol: 10 mM sodium acetate buffer, pH 3.6 = 80: 20: 1), flow rate (α-tocopherol: 0.5 ml/min; β-carotene: 1.0 ml/min.). The detection was performed at 292 nm (α-tocopherol) and 450 nm (β-carotene).

Statistical analysis

The values are expressed as mean ± standard deviation (S.D.). Group mean were compared by a one-way analysis of variance (ANOVA) followed by paired t-test to identify significantly different means between the two groups. Values were considered to be significantly different at $p<0.05$. These statistical analyses were performed with EXCEL Office software on a Windows ME.

RESULTS

Daily nutrients intakes estimated by semiquantitative food frequency questionnaire

Table 1 shows the daily nutrients intakes estimated by semiquantitative food frequency questionnaire in each experimental periods. There were no differences in the energy,

protein, lipids, carbohydrate, cholesterol and fibers during all experimental phases. Also, vitamin E, carotene, and vitamin C intakes did not change during all experimental periods.

Effect of ground green tea drinking on plasma levels of lipids and antioxidants

Total cholesterol and LDL-cholesterol contents in plasma after drinking ground green tea was decreased compared with 0 week, but HDL- cholesterol was not changed in all periods. Triglycerides and TBARS contents in plasma were not changed after ground green tea consumption (table 2). α-tocopherol, β-carotene, uric acid contents and SOD activity in plasma remained unchanged during this study periods. Plasma vitamin C contents was decreased at 2 weeks after drinking ground green tea, but increased at 3 weeks compared with 2 weeks (table 2).

Table 1. Daily nutrient intakes estimated by semiquantitative food frequency questionnaire.

Weeks	-1	0	1	2	3
Drink	Ad libitum.	Water	Green tea		Water
Energy (Kcal/day)	2110±561	2146±361	2023±430	1677±510	1744±481
Protein (g/day)	65.0±15.3	79.2±35.8	62.9±29.3	47.9±13.1	50.1±8.4
Carbohydrate (g/day)	326±115	304±70	313±57	255±86	281±101
Lipids (g/day)	52.8±17.5	58.8±20.5	50.8±23.9	41.2±14.9	40.3±9.3
Cholesterol (mg/day)	261±67	332±157	231±123	238±89	197±79
Fiber (g/day)	11.1±4.4	9.5±1.8	7.8±1.2	7.1±1.6	8.2±1.9
Vitamin E (mg/day)	7.2±2.9	8.1±3.0	7.5±3.5	6.9±1.4	5.6±1.4
Carotene (μ g/day)	1885±1151	1419±647	1859±1733	1735±978	1137±858
Vitamin C (mg/day)	85±95	51±17	46±26	53±19	43±19

Values are mean±S.D. N = 5.

Table2. Effect of ground green tea drinking on plasma levels of lipids and antioxidants.

	Week				
	-1	0	1	2	3
Total cholesterol (mg/dl)	187± 33	178± 36	165± 36†	165± 38‡	164± 43
Trigycerides (mg/dl)	67± 37	84± 42	61± 17	65± 17	75± 57
HDL-cholesterol (mg/dl)	68± 15	63± 15	62± 14	64± 12	63± 15
LDL-cholesterol (mg/dl)	104± 32	100± 33	92± 28	88± 33§	93± 38
TBARS (nmole/ml)	1.64± 0.49	1.56± 0.19	1.48± 0.16	1.44± 0.15	1.58± 0.15
α-tocopherol (mg/dl)	0.73± 0.23	0.72± 0.16	0.73± 0.22	0.78± 0.24	0.81± 0.29
β-carotene (μg/dl)	27.2± 8.9	28.8± 12.8	36.9± 12.6	35.7± 9.9	35.6± 13.7
Vitamin C (μg/ml)	6.2± 1.9¶	3.9± 1.4	4.6± 1.2	2.8± 0.8*	4.6± 0.5※
Uric acid (mg/dl)	5.1± 0.8	4.5± 1.4	5.0± 1.1	4.7± 0.9	4.2± 0.9
SOD activity (%)	14.4± 3.3	15.8± 3.6	17.6± 1.61	15.3± 2.2	13.5± 2.3

TBARS: Thiobarbituric acid reactive substances. SOD: Superoxide dismutase.
†: $p < 0.02$ when compared with 0 week . ‡: $p < 0.003$ when compared with 0 week.
§ : $p < 0.004$ When compared with 0 week. ¶: $p < 0.004$ when compared with 0 week.
＊: $p < 0.02$ when compared with 1 week. ※ : $p<0.02$ when compared with 2 week.
Values are mean±S.D. N=5.

Effect of ground green tea drinking on the lag time of conjugated dienes formation in plasma and LDL

Figure 1 shows that the lag time in conjugated dienes production in plasma by $CuSO_4$ was increased after drinking green tea, but was decreased after the abstention of drinking green tea. The lag time of conjugated dienes formation on LDL oxidation was increased at 1 week after consumption of ground green tea (fig. 1), and decreased after the abstention of drinking ground green tea.

Effects of ground green tea drinking on plasma catechins contents

Figure 2 shows the plasma catechins concentrations after ground green tea consumption. The concentrations of catechins in plasma were not changed after ground green tea consumption.

Effect of ground green tea drinking on α-tocopherol and β-carotene in LDL

Figure 3 shows the effects of ground green tea on α-tocopherol and β-carotene in LDL. The concentrations of α-tocopherol and β-carotene in LDL were not changed after ground green tea consumption.

DISCUSSION

We investigated the effects of ground green tea drinking on the susceptibility of plasma and LDL to the oxidation by $CuSO_4$ *ex vivo* and plasma lipids content in this study. This study demonstrated that the ground green tea consumption for 2 weeks suppressed the susceptibility of plasma and LDL to the oxidation by $CuSO_4$, and resulted the decrease of cholesterol content in plasma and LDL. There are numerous reports about the effects of green tea drinking in human on the susceptibility of plasma and LDL to the oxidation [8, 13, 16, 38]. However, these results are not consistent [8, 13, 16, 38]. Then, we used the ground green tea (pulverized green tea leaves) to increase the amount of consumption of catechins and examined food intakes about each subjects with a semiquantitative food frequency questionnaire during all experimental periods, and guided each subjects about food intakes. The intakes of the nutrients during study were not changed in 3 periods by the nutritional guidance.

There are not always consisted with the reports about the decrease of cholesterol contents in plasma and LDL in human after the ingestion of green tea extracts [5, 23, 28, 38, 44]. For example, van het Hof et al. [38] reported that daily consumption of 900 mL green tea (freeze dried green tea 3g/ 900 ml /day. Total catechins: 21.4 % by wt) for 4 weeks had no effect on serum cholesterol. Princen et al. [28] also showed that healthy smoker consumed freeze dried green tea (3 g/900 ml/day: total catechins: 852 mg/day) for 4 weeks, but green tea consumption had no effects on cholesterol in plasma, LDL and HDL. Freese et al. [5] observed that healthy female volunteers ingested green tea extract powder (3 g/day: total catechins 630 mg/day, other polyphenol 180 mg/day) for 4 weeks, but cholesterol levels in plasma remained constant during the experimental periods. On the other hand, Maron et al. [23] reported that 120 subjects consumed theaflavin-enriched green tea extract (150 mg of green tea catechins, 150 mg of other green tea catechins, and 75 mg of theaflavin) for 12 weeks, and the green tea extracts decreased serum and LDL-cholesterol by 11.3% and 16.4%, respectively. In this study, we showed the decrease of cholesterol contents in plasma and LDL by the consumption of ground green tea (1.5 g/3 times/day). This is in agreement with several previous observations of the experimental animals and human [22, 23, 25]. This may be contributed to the excretion of cholesterol into feces [25], the activities about lipid enzymes [1, 21, 39, 40], the apoB secretion [43] and uptake in cells [3].

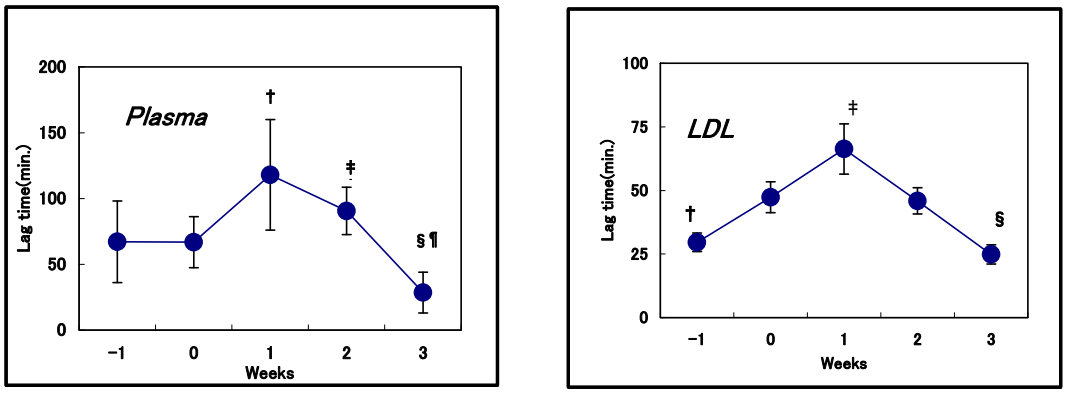

Figure 1. Effect of green tea on the lag time of conjugated dienes formation in plasma and LDL.
Plasma †: 0 Week. VS. 1 Week. (p＜0.03). LDL †: 0 Week. VS. -1 Week. (p＜0.00001).
§: 0 Week. VS. 3 Week. (p＜0.005). ‡: 0 Week. VS. 1 Week. (p＜ 0.003).
‡: 0 Week VS. 2 Week. (p＜0.01). §: 0 Week. VS. 3 Week. (p＜0.00001).
¶: 2 Week. VS. 3 Week. (p＜0.0001). Values are mean±S.D. N＝5.

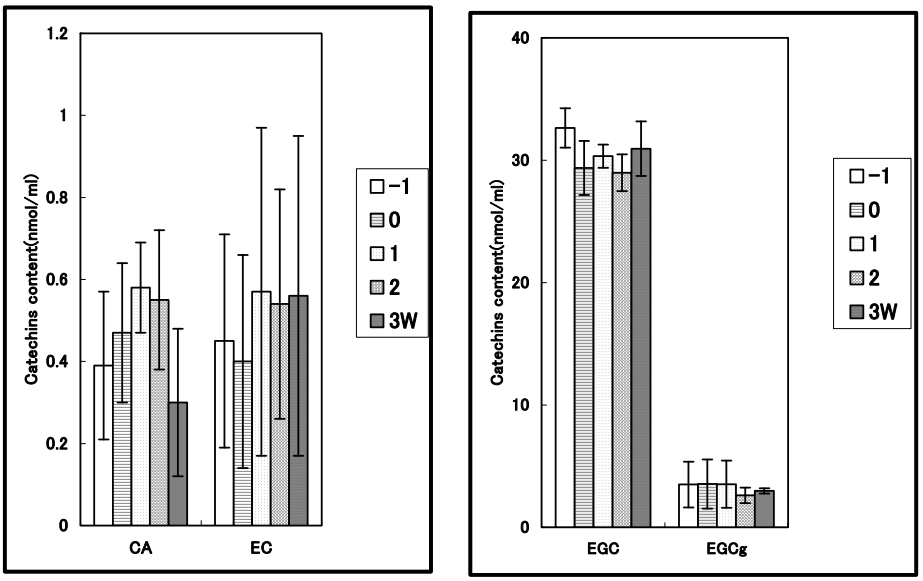

Figure 2. Catechins concentration in plasma after ingestion of ground green tea.
CA: Catechin. EC: Epicatechin. EGC: Epigallocatechin.
EGCg: Epigallocatechin gallate. Values are mean±S.D. N＝5.

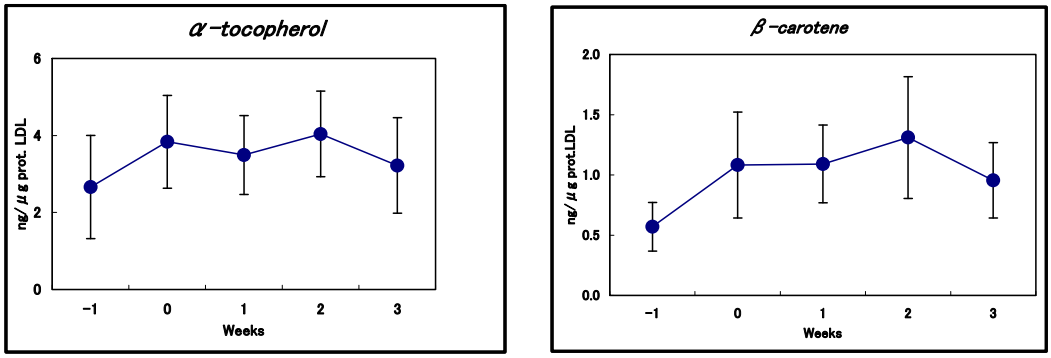

Figure 3. Effect of green tea on α-tocopherol and β-carotene in LDL.
Values are mean±S.D. N=5.

The susceptibility of plasma and LDL to oxidation by $CuSO_4$ was decreased after ground green tea consumption, and increased after the abstention of drinking ground green tea. There are many reports about effects of green tea drinking on the susceptibility of plasma and LDL to the oxidation [8, 13, 16, 38]. However, these reports were not always consistent [8, 13, 16, 38]. In negative reports about effect of green tea drinking on susceptibility of plasma and LDL to oxidation, the quantity of the assimilated green tea might not be enough to body weight. The guidance of the meal ingestion may be insufficient. In this study, all volunteers were young female. The susceptibility of plasma and LDL to oxidation may be affected by estradiol during menstrual cycle. Maziére et al. [24] reported that estradiol inhibited LDL oxidation in the range of concentrations from 5 to 50 μM *in vitro*. However, Ruiz-Sanz et al. [31] reported that the LDL oxidizability indices (lag time before the onset of propagation and the maximal oxidation rate) did not change during menstrual cycles. During follicular phase of high concentration of 17β-estradiol (147.9±88.5 pg/ml, 0.54 nM), the oxidizability did not changed. The concentrations of estrogen required to demonstrate antioxidant activity *in vitro* study exceed levels observed during menstrual cycle. Therefore, it is conceivable that the menstrual cycle did not affect the susceptibility of LDL to oxidation in this study.

In this study, daily consumption of ground green tea for 2 weeks had no effect on serum TBARS. There are reports that lipid peroxide contents in plasma is not changed or decreased after green tea consumption [16, 26]. Nakagawa et al. [26] reported that phosphatidylcholine hydroperoxide levels in human plasma decreased at 60 min after ingestion of green tea extract (total catechins 254 mg). On the other hand, van het Hof et al. [38] reported that green tea consumption for 4 weeks did not affect serum malonaldehyde in human.

We measured the catechins contents in the plasma sampled after 12 h fasting. The concentration of catechins in plasma did not change. We showed in previous report [8] that EGCg and total catechins contents in plasma were increased at 3 h after consumption of ground green tea. Lee et al. [18] reported that the elimination of half lives catechins in a single oral dose of green tea were 1.7-3.4 h. Therefore, it is conceivable that most catechins in blood were excreted in urine [19], and catechins concentrations in plasma did not change in this study. However, catechins may be increased in plasma in several hours after ground green tea consumption, and catechins may be scavenging the oxygen radicals in plasma [8].

In addition to catechins, the ground green tea contained the antioxidants such as α-tocopherol, β-carotene and vitamin C. There was no significant difference in

α-tocopherol and β-carotene concentrations in plasma after ground green tea consumption. Also, there was no significant difference in the intakes of these antioxidant substances during all experimental periods. Vitamin C concentration in plasma at -1 weeks were significantly higher than those at 0 week. It is conceivable that there were the restriction of fruits and its juice from -1 week to 3 weeks. Although the cause about the increase of vitamin C content in plasma from 2 weeks to 3 week is unclear, catechins may reduce the oxidation of vitamin C by active oxygen, and the effects may be delayed. Further study is needed to elucidate the effect of catechins on vitamin C oxidation.

We investigated the effects of ground green tea drinking on uric acid and SOD activity in plasma, but the consumption of ground green tea did not significantly influence uric acid and SOD activity in plasma. These findings are consisted with other reports [28, 38]. It is well known that α-tocopherol, β-carotene and cholesterol in LDL have effect of the sensitivity of LDL to the oxidation [6, 20, 29]. Frei and Gaziano et al. [6] found that an increased cholesterol content of LDL was associated with an increased oxidation susceptibility of the lipoprotein. Total cholesterol contents in LDL after drinking ground green tea decreased compared with 0 week in this study. Therefore, the changes of susceptibility of LDL to oxidation after the ground green tea consumption may be due to the changes of cholesterol contents rather than α-tocopherol and β-carotene in LDL.

In conclusion, the ground green tea consumption for 2 weeks results the decrease of cholesterol content in plasma and LDL, and suppressed the susceptibility of plasma and LDL to the oxidation by $CuSO_4$. The further study is mandatory to elucidate the mechanisms.

REFERENCES

1. **Abe, I., Seki, T., Umehara, K., Miyase, T., Noguchi, H., Sakakibara, J., and Ono, T.** 2000. Green tea polyphenols: Novel and Potent inhibitors of squalene epoxidase. Biochem. Biophys. Res. Commun **268**: 767-771.
2. **Abe, K., Yuguchi, Y., and Katsugi, G.** 1975. Quantitative determination of tocopherol by high-speed liquid chromatography. J. Nutr. Sci. Vitaminol **21**: 183-188.
3. **Bursill, C., Roach, P.D., Bottema, C.D.K., and Pal, S.** 2001. Green tea upregulates the low-density lipoprotein receptor through the sterol-regulated element binding protein in HepG2 Cells. J. Agric. Food Chem **49**: 5639-5645.
4. **Esterbauer, H., Strieg H., Puhl, H., and Rotheneder, M.** 1989. Continuous monitoring of *in vitro* oxidation of human low density lipoprotein. Free. Radic. Res. Commun **6**: 67-75.
5. **Freese, R., Basu, S., Hietanen, E., Nair, J., Nakachi, K., Bartsch, H., and Mutanen, M.** 1999. Green tea extract decreases plasma malondialdehyde concentration but dose not affect other indicators of oxidative stress, nitric oxide production, or hemostatic factors during a high-linoleic acid diet in healthy females. Eur J Nutr. **38**: 149-157.
6. **Frei, B., and Gaziano, J.M.** 1993. Content of antioxidants, preformed lipid hydroperoxides, and cholesterol as predictors of the susceptibility of human LDL to metal ion-dependent and -independent oxidation. J. Lipid Res. **34**: 2135-2145.
7. **Geleijinse, J.M., Launer, L.J., van der Kuip., M., Hofman, A., and Witteman, J.C.W.** 2002. Inverse association of tea and flavonoid intakes with incident mycardial infarction: the Rotterdam Study. Am. J. Clin. Nutr. **75**: 880-886.
8. **Gomikawa, S., and Ishikawa, U.** 2002. Effects of catechins and ground green tea drinking on the susceptibility of plasma and LDL to the oxidation *in vitro and in vivo.* J. Clin. Biochem. Nutr. **32**: 55-68.
9. **Graham, H.N.** 1992. Green tea composition and polyphenol chemistry. Prev. Med. **21**:

334-350.
10. **Graham, F.M., Maureen, L., and Philip, W.C.** 1989. Sodium dodecyl sulfate-glycerol polyacrylamide slab gel electrophoresis for the resolution of apolipoproteins. J. Lipid Res. **30**: 757-761.
11. **Han, D.W., Lim, H.R., Baek, H.S., Lee, M.H., Lee, S.J., Hyon, S.H., and Park, J.C.** 2006. Inhibitory effects of epigallocatechin-3-o-gallate on serum-stimulated rat aortic smooth muscle cells via nuclear factor-κB down-modulation. Biochem. Biophys. Res. Commun **345**: 148-155.
12. **Hertong, M.G.L., Feskens, E.J.M., Hollman, P.C.H., Katan, M.B., and Kromhout, D.** 1993. Dietary antioxidant flavonoids and risk of coronary heart disease: the Zutphen Elderly Study. Lancet. **342**: 1007-1011.
13. **Hodgson, J.M.,Croft, K.D., Mori, T.A., Burke, V., Beillin, L.J., and Puddey, I.B.** 2002. Regular ingestion of tea dose not inhibit in vitro lipid peroxidation in humans. J. Nutr. **132**: 55-58.
14. **Kamiyama, S., Tajika, K., and Ito, Y.** 1987. The determination of carotene. Rinsho Kensa **31**: 268-274.
15. **Knekt, P., Javinen, R., Reunanen, A., and Maatela, J.** 1996. Flavonoid intake and coronary mortality in Filand: cohort study. Brit Med J. **312**: 478-481.
16. **Koga, T., and Meydani, M.** 2001. Effect of plasma metabolism of (+) catechin and quercetin on monocyte adhesion to human aortic endothelial cells. Am. J. Clin. Nutr. **73**: 941-948.
17. **Kontush, A., Spranger, T., Reich, A., Djahansouzi, S., Karten, B., Braesen, J.H., Finckh, B., Kohlshutter, A., and Beisiegel, U.** 1997. Whole plasma oxidation assay as a measure of lipoprotein oxidizability. Biofactor **6**: 99-109.
18. **Lee M.-J., Maliakal, P., Chen, L., Meng, X., Bondoc, F.Y., Prabhu, S., Lambert, G., Mohr, S., and Yang, C.S.** 2002. Pharmacokinetics of tea catechins after ingestion of green tea and (-)-epigallocatechin-3-gallate by humans: Formation of different metabolites and individual variability. Cancer Epidemiol. Biomark. Prev. **11**: 1025-1032.
19. **Lee M-J., Wang , Z.Y., Li, H., Chen, L., Sun, Y., Gobbo, S., Balentine, DA., and Yang, CS.** 1995. Analysis of plasma and urinary tea polyphenols in human subjects. Cancer Epidemiol. Biomark. Prev. **4**: 393-399.
20. Levy, Y., Kaplan, M., Ben-Amotz, A., and Aviram, A. 1996. Effect of dietary supplementation of beta-carotene on human monocyte-macrophage-mediated oxidation of low density lipoprotein. Isr J Med Sci **32**: 473-478.
21. **Lin, J.C., Chan, P., Hsu, F.L., Chen, Y.J., Hsieh, M.H., Lo, M.Y., and Lin, JY.** 2002. The in vitro inhibitory effects of crude extracts of traditional Chinese herbs on 3-hydroxy-3-methylylutaryl- coenzyme A reductase on Vero cells. Am J Chin Med **30**: 629-636
22. **Loest, H.B., Noh, S.K., and Koo, S.I.** 2002. Green tea extract inhibits the lymphatic absorption of cholesterol and α-tochopherol in ovariectomized rat. J. Nutr. **132**: 1282-1288.
23. **Maron, D.J., Lu, G.P., Cai, N.S., Wu, Z.G., Li, Y.H., Chen, H., Zhu, J.Q., Jin, X.J., Wouters, B.C., and Zhao, J.** 2003. Cholesterol -lowering effect of a theaflavin-enriched green tea extract. randomized controlled trial. Arch Intern Med **163**: 1448-1453.
24. **Mazière, C., Auclair, M., Ronveaux, MF., Salmon, S., Santus, R., and Mazière, JC.** 1991. Estrogens inhibit copper and cell-mediated modification of low density

lipoprotein. Arteriosclerosis **89**: 175-182.

25. **Muramatsu, K., Fukuyo, M., and Hara, Y.** 1986. Effect of green tea catechin on plasma cholesterol levels in cholesterol-fed rats. J. Nutr. Sci. Vitaminol **32**: 613-622.
26. **Nakagawa, K., Ninomiya, M., Okubo, T., Aoi, N., Juneja, L.R, Kim, M., Yamanaka, K., and Miyazawa, T.** 1999. Tea catechin supplementation increases antioxidant capacity and prevents phospholipid hydroperoxidation in plasma of humans. J. Agric. Food Chem **47**: 3967-3973.
27. **Negishi, H., Xu, J.W., Ikeda, K., Njelkela, M., Nara, Y., and Yamori, Y.** 2004. Black and green tea polyphenols attenuate blood pressure increases in stroke-prone spontaneously hypertensive rats. J. Nutr **134**: 38-42.
28. **Princen, H.M.G., van Duyvenvoorde W., Buytenhek, R., Blonk, C., Tijburg , L.B.M., Languis, J.A.E., Meinders, A.E.M., and Pijl, H.** 1988. No effect of consumption of green and black tea on plasma lipid and antioxidant levels and on LDL oxidation in smokers. Arterioscler Thromb Vasc Biol **18**: 833-841.
29. **Princen, H.M.G., van Duyvenvoorde W., Buytenhek, R., van der Laarse A., van Poppel G., Leuven, J.A.G., and van Hinsbergh, V.W.M.** 1995. Supplement with low dose of vitamin E protects LDL from lipid peroxidation in men and women. Arterioscler Thromb Vasc Biol **15**: 325-333.
30. **Rimm, E.B., Katan, M.B., Ascherio, A., Stampfer, M.J., and Willett, WC.** 1996. Relation between intake of flavonoids and risk for coronary heart disease in male health professional. Ann Intern Medicine **125**: 384-389.
31. **Ruiz-Sanz, JI., Navarro, R., Martínez, R., Hernández, ML., Matorras, R., and Ruiz-Larrea, MB.** 2007. No effect of menstrual cycle on LDL oxidizability and particle size. Maturitas **57**: 253-260.
32. **Serafini, M., Ghiselli, A., and Ferro-Luzzi, A.** 1996. In vivo antioxidant effect of green and in black tea in man. Eur. J. Clin. Nutr **50**: 28-32.
33. **Shidouji, Y.** 1990. Vitamin handbook. Kagakudoujin Press, Tokyo, pp. 27-35.
34. **Speek, A.J., Schrijver, J., and Schreurs, W.H.** 1984. Fluorometric determination of total vitamin C in whole blood by high-performance liquid chromatography with pre-column derivatizaion. J. Chromatogr **305**: 53-60.
35. **Suzuki, K., Sasagawa, T., Okita, M., Fujiwara, Y., Mori, K., Saito, M., and Abe, Y.** 1996. Development of new simplified -food frequency method for use in the surveillance on life style in order to promote healthy condition in okayama prefecture, computed by Microsoft excel. Bull. Facul. Health & Welfare Sci., Okayama Pref. Univ . **31**: 143-153.
36. **Tokunaga, S., White I, Frost. C., Tanaka, K., Kono, S., Tokudome, S., Akamatu, T., Moriyama, T., and Zakouji, H.** 2002. Green tea consumption and serum lipids and lipoproteins in a population of healthy workers in japan. Ann Epidemiol **12**: 157-165.
37. **Tubono, Y., and Tugane, S.** 1997. Green tea intake in relation to serum lipid levels in middle-aged J apanese men and women. Ann Epidemiol **7**: 280-284.
38. **van het Hof KH., de Boer HSM., Wiseman, S.A., Lien, N., Weststrate, J.A., and Tijburg,L.B.** 1997. Consumption of green or black tea dose not increase resistance of low-density lipoprotein to oxidation in humans. Am. J. Clin. Nutr **66**: 1125-1132.
39. **Wang, X., and Tian, W.** 2001. Green tea epigallocatechin gallate : A natural inhibitor of fatty acid synthase. Biochem. Biophys. Res. Commun **288**: 1200-1206.
40. **Watanabe, J., Kawabata, J., and Niki, R.** 1998. Isolation and identification of acetyl-CoA carboxylase inhibitors from green tea (Camellia sinensis). Biosci Biotechnol Biochem **62**: 532-534.

41. **Yagi, K.** 1976. A simple fluorometric assay for lipoperoxide in blood plasma. Biochem. Med **15**: 212-216.
42. **Yamanaka, N., Oda, O., and Nagao, S.** 1997. Green tea catechins such as (-) epicatechin and (-) epigallocatechin accelerate Cu^{2+}-induced low density-lipoprotein oxidation in propagation phase. FEBS Lett **401**: 230-234.
43. **Yee,W., Wang, Q., Agdinaoay, T., Dang, K., Chang, H., Grandinetti, A., Franke, A.A., and Theriault, A.** 2002. Green tea catechins decrease apolipoprotein B-100 secretion from $HepG_2$ cells. Mol Cell Biochem **229**: 85-92.
44. **Young, J.F., Dragstedt, L.O., Haraldsdottir, J., Daneshvar, B., Kall, M.A., Loft, S., Nilsson, L., Nilsson, S.E., Mayer, B., Skibsted, L.H., Huynh-Ba, T., Hermetter, A., and Sandstrom, B.** 2002. Green tea extract only affects markers of oxidative status postprandially: lasting antioxidant effect of flavonoid-free diet. Brit J Nutr **87**: 343-355.